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THE ROLE OF NUCLEIC ACIDS IN HEREDITY AND VARIABILITY OF MICROORGANISMS

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The problem of the role of the nucleic acids in heredity and variability of microorganisms occupies a central position in microbiology and particularly in the problem of bacterial genetics. Furthermore, investigations on the study of the genetic role of the nucleic acids made on microorganisms serve as the basis for the general biological conclusions of the essence and mechanism of heredity and of its variability.

These investigations have received special attention after work on the transforming activity of desoxyribonucleic acid (DNA).

As is well known, following the intiial work of Avery and others on the transformation of pneumococci, numerous investigations were carried out for reproducing the phenomenon of transformation in different species of microorganisms (hemophilic influenza bacteria, causal organisms of dysentery, colon bacilli, bacteria of avine tuberculosis, meningococci, staphylococci, etc.), in which the possibility was shown of transmission of more than 30 features from some microorganisms to others. On a model of pneumococci and hemophilic influenzal bacteria individual aspects of the transformation reaction were studied: the significance of the selection of "donor" strains and "recipient" strains, the significance of conditions of the medium in which the culture to be altered was grown, various methods of obtaining and purifying the transforming agent, etc. (see the reviews of Timakov and Skavronskaya, 1956; Loginova, 1957).

In subsequent years, a number of new investigations have been published on the study of the phenomenon of transformation (Hotchkiss, 1955, 1956, 1957; Ephrussi-Taylor, 1955,

1957, Zamenhof and others, 1956; Leidy and others, 1956; Drew, 1957; Schaeffer, 1957; Goodgal and Herriott, 1957; Thomas, 1957).

In these investigations numerous facts were obtained, which attest, without doubt, to the genetic role of DNA, and although in transformation experiments the possibility of transmission of only individual features has been established (basically, type-specificity and resistance to antibiotics), desoxyribonucleic acid began to be regarded as the single determinant of heredity, responsible for all the properties of the bacterial cell.

Careful analysis of the published material and an objective evaluation of it with consideration of the results of investigations of other rules and regulations observable in the process of variability do not give us the bases for accepting the exclusive and single role of DNA in the determination of hereditary features. The theory of the identity of DNA and the gene is also unproved. This is why many investigators, even among those who are working out the problem of heredity and variability in the aspect of conceptions of formal genetics, while recognizing the genetic significance of DNA, are revising the theory of its exclusive genetic role which was postulated after the first investigations on the transformation of the microorganisms (Zamenhof, 1957; Chargaff, 1957; Ephrussi-Taylor, 1955).

Let us see which experimental data confirm the conception of DNA as the basic and even the only factor in heredity, and which facts contradict this conception.

As has already been stated, the most convincing facts attesting to the significance of DNA in heredity and variability were the investigations on the transformation of the microorganisms. In these investigations it was shown that by means of DNA it is possible to transmit hereditarily reinforced features and properties from one serological type of microbe to another. Afterwards, it was established that DNA produces a transformation of the microorganisms in very small numbers. Its activity does not decrease with the purification of the preparation with respect to protein and ribonucleic acid and is completely lost after the action of desoxyribonuclease.

In contrast to the data on DNA as the basic factor controlling the various hereditary features of microorganisms, investigations have been made which attest to the identity

of the chemical properties of the desoxyribonucleic acids isolated from the various microorganisms as well as from the various organs and tissues of animals and man.

This contradiction, however, has, at the present time, been explained by a working hypothesis proposed by Watson and Crick, dealing with a model of the structure of DNA molecules and a number of investigations performed for the elucidation of the specificity of DNA molecules.

According to the model of Watson and Crick, the DNA molecules are represented in the form of two chains consisting of alternating sugars and phosphates; a purine or pyramidine base is attached to each sugar unit. The chains are twisted spirally around the general axis and connected with one another by internucleotide hydrogen bonds. Here, there is a strict specificity in the pairing of the bases: adenine is connected with thymine, guanine with cytosine, 5-methylcytosine or 5-oxymethylcytosine. The sequence of pairs of bases may be entirely different. The character of this sequence conditions the specificity of the molecules, their heterogeneity in the presence of the same chemical composition, and assures the transmission of the information.

A number of aspects of the Watson and Crick hypothesis have been confirmed by experimental data of roentgen-structural analysis, electron microscopy of the DNA filaments, etc. (Watson, 1955; Fraser and Williams, 1953; Williams, 1952; Crick, 1957).

The pairing of the base connections in the DNA molecules is confirmed by the relationship of the quantity of individual purine bases, characteristic of the desoxyribonucleic acids, or the relationship of their sum to the corresponding pyridine bases, which, as a rule, is equal to unity (adenine/thymine = 1; guanine/cytosine = 1; adenine plus guanine/thymine + cytosine = 1).

If the position of Watson and Crick is correct, according to which the biological specificity of DNA is determined the character of the arrangement of purine and pyrimidine bases in its molecules, an experimentally produced alteration of the nucleotide composition of the DNA of live cells should appear in a change of the hereditary properties of the microorganisms.

Based on this premise, Zamenhof and others (1954, 1956)

carried out investigations on colon bacilli, replacing the pyrimidine base of DNA thymine with its structural analogues 5-bromuracil, 5-chloruracil and 5-iodouracil. The changes observed in the experiments could not be considered genotypic.

In culturing microorganisms on media containing these substances, 18-20 percent of the DNA thymine was replaced by its analogues, which was considered by the authors as a marked alteration of the suggested heredity determinant. However, only reversible changes in the colony morphology and form of the cells occurred, which were maintained for several transplantations. The authors explained this by the fact that the assimilation occurred only in genetically inactive molecules.

In the experiments of Dunn and Smith (1954) performed on bacteriophages T_2 and T_6, it was shown that the phages containing 5-iodouracil and 5-bromuracil were indistinguishable from the normal on electron microscopy. However, 50-90 percent of the particles lose their infecticusness.

For the purpose of proving the possibilities of genetic control of the various features of the bacterial cells by desoxyribonucleic acid the molecular specificity of DNA is being studied, that is, the heterogeneity of the molecules in a sample of DNA isolated from a single source, including in biologically active preparations of desoxyribonucleic acid. Thus, by the fractionation of DNA samples obtained from various tissues and subsequent determination of the nucleotide composition of it the heterogeneity of the fractions obtained has been established.

Brown and Watson (1953) published investigations showing the lack of homogeneity of DNA molecules of E. coli, of calf thymus and of red blood cells of man with respect to their interaction with histone. The authors carried out the artificial adsorption of DNA on histone, and then washed it out with NaCl solution of different concentrations. Hereby, fractions were obtained, in which the interrelationship of the bases varied depending on the concentration of the NaCl solution.

The same method was used by Brown and Martin (1955) for the analysis of the DNA of bacteriogphage T_2, in the composition of which two fractions were found. Fraction A, containing about 30 percent of the total nucleic acid phosphorus with a relationship of $\frac{\text{adenine + thymine}}{\text{quanine + oxymethylcytesine}} = 1.9$,

and fraction B, containing 70 percent of the total phosphorus, where the ratio mentioned above was equal to 2.15.

According to the data of Bendich, Russel and Brown (1953), in DNA preparations obtained from organs of rats, two fractions were obtained which were different in their solubilities in 0.85 percent NaCl. The interrelationship of these fractions in the DNA of different organs varies.

In the study of the DNA of the colon bacillus cultured on a medium with tagged 5-bromuracil, Bendich, Pahl and Brown (1957) established the heterogeneity of it with respect to the incorporation of this pyrimidine.

Lerman (1955) subjected the transforming factor extracted from pneumococci to fractionation. By means of chromatography he established the heterogeneity of this factor and obtained homogeneous fractions preserving their chromatographic identity on repeated fractionation.

Marmur and Fluke (1955) attempted to show differences in the molecules of biologically active DNA of pneumococci by means of ionization with electrons. They made use of desoxyribonucleic acid isolated from a strain possessing four tagged features: the capacity of utilizing mannitol, resistance to penicillin, streptomycin and sulfonamides. After electronic bombardment of the DNA samples and subsequent testing of their transforming activity, no differences were observed in the frequency of transformations of the individual features. Only a parallel reduction of transforming activity was noted with respect to all the features, the degree of which depended on the dose of electrons per square centimeter.

The data presented above are unconditional evidence of the presence of different fractions in the composition of DNA preparations isolated from the same source. However, they are still inadequate for proving the possibility of controlling the infinite variety of properties and features of microorganisms by desoxyribonucleic acid.

Another type of specificity of nucleic acids is species specificity. In the study of the desoxyribonucleic acids isolated from the organs and tissues of various species, Chargaff and others established their species specificity with respect to their nucleotide content (1950, 1953, 1955, 1957).

The investigations of Ki Yong Lee, Wahl and Barbu (1956)

and of Spirin and co-authors (1956,1957) attest to the species specificity of DNA of bacteria. In these investigations it was shown that the relationship $\frac{\text{guanine + cytosine}}{\text{adenine + thymine}}$ varies in distantly related species of microorganisms, whereas there are slight differences in closely related species.

Thus, according to the data of Spirin (Belozerskiy, 1957), the relationship $\frac{g + c}{a + t}$ in Clostridium perfringens is 0.45; in Staphylococcus pyogenes aureus, 0.53; in Proteus vulgaris, 0.68; in Salm. typhi murium, 1.18; in M. tuberculosis BCG, 2.08; and in Actinomyces globisporus Streptomycini, 2.73.

Within the limits of the bacteria of the colon-typhoid group the nucleotide content of the DNA of bacteria of various species was very similar; the relationship indicated above was equal to 1.09 in E. coli, 1.15 in Shigella dysenteriae, 1.14 in Salm. typhosa, and 1.18 in Salm. typhi murium.

The data concerning the chemical differences in desoxyribonucleic acids of certain bacteriophages are interesting. It has been established that DNA of phages T_2, T_4 and T_6 contains 5-oxymethylcytosine instead of the pyrimidine cytosine base characteristic of the other desoyribonucleic acids. In the nucleotides of the DNA of these bacteriophages glucose is found (Cohen, 1956; Wyatt and Cohen, 1953; Volkin, 1954; Sinsheimer, 1954; Asaitis, 1957).

The investigations of Blix, Iland and Stacey attest to the serologic specificity of DNA (1954).

DNA isolated from thymus proved to be highly antigenic when injected into rabbits. The antiserum obtained gave only weak cross-reactions with certain DNA preparations isolated from other sources.

The data on the species specificity of DNA acquire great significance in the study of the change of the nucleotide content of DNA in the process of variability in comparison with the change of the biological properties of the microorganisms. Chargaff established that the nucleotide composition of different variants of one species of microorganisms was constant (1952). Investigations carried out at the Institute of Epidemiology imeni Gamalei and in the department of plant biochemistry of Moscow State University attest to the fact that the alteration of the biological properties

and antigenic composition of microorganisms correlates with the change of the nucleotide content of their DNA (Timakov and others, 1955; Belozerskiy and others, 1955; Timakov, 1958; Spirin and others, 1958 .

Thus, the base-forming variants obtained from the bacteria of the colon group are markedly different in their biologic properties, chemical composition and antigenic structure from the original cultures, and have a markedly different nucleotide composition of the DNA: $\frac{g + c}{a + t}$ in the alkaligenous bacteria was equal to 2.03; in the colon bacillus (original culture), 1.10. No notable changes in RNA content were found here. The nucleotide content of the paratyphoid-like variant obtained from the alkali-former under the influence of killed Breslau paratyphoid bacteria was similar to that of the directing culture but did not coincide with the latter (1.24 in the paratyphoid-like organism and 1.18 in paratyphoid organisms), which is in agreement with certain differences in the biological properties of the paratyphoid-like variant and of Breslau bacteria.

The species specificity of DNA is of indubitable interest. However, one cannot help agreeing with Hershey (1956) that the proof of this kind of specificity only places DNA in a line with other specific substances such as proteins, polysaccharides, etc. and does not attest to its preferential genetic role.

In recent years, the attention of many investigators occupied in the study of the genetic role of the nucleic acids has been attracted to the virus-bacteriophages which are regarded as sources of a relatively pure genetic substance. The investigations of Hershey and Chase published in 1952 contributed to this; here, it was shown in a number of precise and clever experiments that in phage infection the protein membrane of the virus remains outside of the bacterial cell, while the process of reproduction of it is induced by the desoxyribonucleic acid introduced into the bacterium.

In addition, investigations on the study of the process of reproduction of particles of phage are being conducted with the aim of clarifying the mechanism of the phenomenon of transduction. This phenomenon is reproduced by means of certain bacteriophages and is regarded by the majority of investigators as a consequence of the transfer of DNA from one host to another, that is, as a transformation reaction in which the bacteriophage contributes to the penetration

of the transforming agent into the bacteria.

Hershey and Chase (1952) confirmed the data of Anderson and others (1953), attesting to the possibility of a division of phage particles into protein membranes and DNA, liberated into the medium through osmotic shock.

Under conditions where the microorganisms were subjected to marked mechanical effects at the beginning of the latent period and to subsequent centrifugation, the basic quantity (80 percent) of the protein of the phage membranes was found in the sediment, which did not interfere with the formation of a normal phage progency.

In experiments on the adsorption of the phage on to bacterial "membranes" obtained by means of the preliminary destruction of bacteria, DNA was poured directly into the medium, and the protein membranes were precipitated together with the bacteria in the process of centrifugation.

These data permitted the authors to assert that after the adsorption of the phage the DNA of the virus is introduced into the cell, after which its protein membrane has no significance.

Puck and Lee (1955) came to the same conclusions. In the process of penetration of the cell wall by bacterial virus they distinguished the following stages: reversible electrostatic attachment, splitting-off of the DNA of the virus from the protein, the beginning of the lytic reaction at the site of attachment of the virus, the injection of the DNA of the virus into the opening formed in the wall of the bacterial cell, the spread of the lytic reaction to the entire cell surface, the closure of the opening and the decrease of cellular permeability.

The mechanism by means of which the nucleic acid is injected into the cell has not been clarified.

It has been established that the protein membrane of the phage has four different components: the protein of the head, the protein of the proximal part, of the tail, and the protein of the distal (terminal) part of the tail and the inner rod of the tail. The protein nature of the fourth component has not been shown; however, the desoxyribonuclease does not produce changes in its structure (Kellenberger and Arker, 1955).

The phage is adsorbed by the terminal part of the tail; the lytic activity is most marked in the proximal portion. The terminal part can be removed through the action of metals of the zinc group in the presence of cyanide and certain other substances; amino acids, proteins and others (Kozloff and Henderson, 1955; Kozloff, 1956). Here, the upper part of the membrane of the tail is contracted, as a result of which the rod is laid bare. It is assumed that such a disruption of the protein connections in the terminal part of the tail occurs in the process of injection of the DNA into the bacterial cell, in the membrane of which the above mentioned ions are found (Barrington and Kozloff, 1956; Brown and Kozloff, 1957). In this process, Evans (1956) distinguished four periods: adsorption, removal of the distal part of the tail, lysis of the wall of the bacterial cell at the site of attachment of the phage, injection of the inner rod of the tail into the cell together with the DNA.

It is not known whether the rod contributes mechanically to the injection of the nucleic acid or carries the chemically active substances within itself.

The penetration of a certain part of the protein into the infected bacterium was shown in the work of Hershey and Chase (1952). In the later investigations of Hershey (1956) it was shown that in the substrate obtained through the osmotic shock of bacteriophage T_2, apart from the phage membranes identifiable microscopically and possessing a definite speed of precipitation, the capacity of being specifically adsorbed onto the bacteria, being precipitated by the antiphage serum and killing the corresponding microorganisms, there is found one to four percent of non-precipitable protein, which does not correspond to the characteristics of protein membranes. In infection, this protein is injected into the bacterial cells, which in consideration of a number of other factors attesting to the possible significance of protein components in the process of DNA synthesis raises a problem with respect to the assertion of its exclusive genetic significance in the process of reproduction of the bacteriophage.

As proof of the presence of the genetic determinants and the determination of their nature the process is being studied of the transfer of the individual components of the initial particles of the phage to the newly formed progeny.

It is well known that in the latent period the phage particles infecting the cells are subjected to destruction.

Even with a great multitude of infections (seven particles of the phage per single bacterium), artificially lysing the cells at various intervals of the latent period it is not possible to find initial particles of the phage (Anderson and Derman, 1952; Derman, 1952). The DNA of the phage is a component part of the organized structures of the bacterial cell over the course of the latent period, is not liberated in the artificial disruption of the cells and is precipitated with the bacterial "membranes" (Hershey and Chase, 1952).

In the infection of E. coli with T_6 phage tagged with P^{32}, a great part of the phosphorus of the nucleic acid of the original particles in the untagged medium is found in the medium in the form of low molecular-weight unprecipitable phosphorus, but 22-42 percent is transmitted to the phage progeny (Putnam and Kozloff, 1951).

Similar results with respect to the transfer of phosphorus were obtained by Graham and Hershey and others (Graham, 1953).

Graham established the fact that almost the entire phosphorus is contained in the newly formed particles of phage, while 85 percent of the progeny do not contain the parent phosphorus at all or contain very slight amounts of it. This has made it possible for him to draw the conclusion that the transmission of the phosphorus (that is, the nucleic acid phosphorus) is not necessary for the transfer of the hereditary properties.

Levinthal (1956), by means of microscopy of traces left in highly sensitive photographic film by individual radioactive phage particles tagged with P^{32} showed that the DNA of each particle consists of one large and many small structures. In his opinion, the large structure is divided into no more than two parts and is transmitted by the first two particles of the newly formed bacteriophages, while the small ones are distributed among the many daughter particles, nuclei of which are copied in the course of reproduction from the large particle of the initial phage.

Levinthal's data attest to the possible genetic role of DNA of the phage in the absence of a correlation between the transfer of the hereditary properties of the newly formed particles and a direct transmission of the phosphorus to the progeny.

According to the investigations of Kozloff (1952), trans-

mission of the DNA particles to the progeny occurs through the products of decomposition rather than directly. The difference in the interrelationship of the tagged phosphorus and nitrogen in the original and newly formed bacteriophages serves as proof of this.

Concerning the protein components it is well known that in the experiments with bacteriophages tagged with sulfur, an insignificant part (less than one percent) of the sulfur-containing protein of the original particles is transmitted to the progeny. The possibility of the transfer of protein which does not contain sulfur has not been clarified.

Therefore, at the present time it is impossible to consider precisely established the presence of genetic units transmitted to the phage progeny nor, particularly, chemically identifying them.

In the infection of bacteria, a much larger quantity of nucleic acid is injected into them than protein (each particle of phage injects 2×10^{-10} gamma of DNA and 1/10 of protein). The quantity of these components in the mature bacteriophages is approximately equal (40-50 percent DNA and 50-60 percent of protein). This speaks for the greater importance of nucleic acid for the process of reproduction of the phage, but does not attest to the autonomy of its synthesis.

Experiments with synthesis inhibitors as well as experiments on microorganisms which need individual amino acids and which are not capable of synthesis of proteins in the absence of these compounds are contributing to the elucidation of the problem of the independent formation of DNA. (Hershey, 1956; Hershey and Melechen, 1957; Barton, 1955, 1956; Tomitsawa and Sunikawa, 1956; Rosenbaum and others, 1955).

In experiments with definite bacteriophages it has been shown that the DNA of the phage containing 5-oxymethylcytosine is found in the infected cell as early as three minutes after the infection. The phage protein is synthesized somewhat later. However, for the purpose of beginning the synthesis of the nucleic acid of the virus, conditions are needed for assuring the possibility of the synthesis of the protein of the bacterial cell.

Thus, in the infection of the auxotrophic variants of E. coli in the medium deprived of aminoacids corresponding to

their requirements, DNA of the phage is not formed. The same effect was obtained after the addition of 5-methyl tryptophase to the infected culture, which is an antimetabolite and prevents the formation of tryptophane.

The addition of chlormycetin and chloramphenicol (inhibitors of protein synthesis) in the first few minutes after the infection also depresses the formation of the phage DNA.

Protein synthesis has importance only for the onset of the formation of DNA (first five to ten minutes). Later, it may be stopped without harm to the synthesis of nucleic acid.

The nature and function of protein, the formation of which is needed for the beginning of DNA synthesis, has not been clarified. It is supposed that the enzymes formed in its composition condition the synthesis of nucleic acid of the phage. In the opinion of Hershey, probably this protein is the pattern for determining the sequence of the nucleotides in the molecules of the newly formed DNA. The genetic role in such a case belongs to the protein.

It has been established in a number of investigations that in the artificial lysing of infected bacteria at the end of the latent period the components of the phage particles (protein and nucleic acid) are found separately. The predecessors of the membranes, which have received the name of "doughnuts", practically contain no phosphorus, that is, they have no nucleic acid (De Mars, Luria, Fischer, Levinthal, 1953). It is not known whether a separation of the components occurs in the course of the processing or whether the phage predecessors are formed separately. In principle, the question is important as to which of these predecessors is formed first. Their onsets of formation are only very slightly different in time.

Melechen and Hershey carried out investigations with the aim of elucidating whether DNA of phage can be formed without considerable formation of phage protein. Thereby, chloromycetin, was added to the infected bacteria seven minutes after the infection; it suppressed the synthesis of protein. At 45 minutes, the chloromycetin was removed and the culture was again suspended in the medium (Hershey, 1956).

During the time the infected culture was in the medium with the chloromycetin 100 phage-equivalent units of DNA were formed and, at most, 10 phage-equivalent units of pro-

tein; the major part of the latter was synthesized before the addition of the chloromycetin. After the removal of the chloromycetin, the formation of normal phage particles was noted.

If the DNA formed after the removal of the chlormycetin (that is, DNA formed under conditions of normal protein synthesis) is tagged with P^{32}, and then the content of nucleic acid formed in the presence of chlormycetin and after its removal is determined in the mature phage particles, it is shown that they contain no more than one percent tagged DNA.

These data speak for the fact that chlormycetin and postchlormycetic acid when injected into the protein membrane complete on an equal basis, which attests to the normal functional capacity of the desoxyribonucleic acid formed without the simultaneous synthesis of protein, and, therefore, also to the separate formation of DNA and protein in the process of reproduction of the bacteriophage. However, the data presented above, in the opinion of the authors, still do not prove the genetic role of DNA, because the significance of the protein component associated with the nucleic acid in the terminal phase of formation of the phage remains unclear, as does also the significance of the protein the formation of which, as has been mentioned above, must of necessity precede the synthesis of nucleic acid.

Investigations on the study of the possibility of transfer of DNA particles from some microorganisms to others by means of bacteriophages have been devoted to the elucidation of the role of desoxyribonucleic acid in the heredity and variability of microorganisms. Suppositions concerning the possibility of such a transfer are based on data concerning the phenomenon of transduction which has many features in common with the reaction of transformation, which arises through the direct action of DNA on the bacterial cell.

The first investigations on the reproduction of the phenomenon of transduction were published by Lederberg, Lederberg, Zinder and Lively (1951). Their experiments were carried out on salmonellae of 22 phagotypes, the diauxotrophic variants of which were used in the capacity of the original cultures. After the cultivation of two auxotrophs needing various substances in a mixed culture and subsequent plating out of them on medium deprived of the given sources of nutrition, the growth of prototrophs was observed.

In the subsequent experiments an active transforming ex-

tract was obtained through the action of mild-acting bacteriophage isolated from the lysogenic culture. After the action on auxotrophs of phage lysate obtained through the cultivation of phage on a culture of prototrophs, the auxotrophic variants acquired the capacity of growing in a medium deprived of a source of organic nitrogen.

Zinder and Lederberg (1952, 1953, 1955) carried out investigations on a directed alteration of the fermentative activity, antigenic structure, and sensitivity to antibiotics of salmonellae by means of mild bacteriophage.

In the work of Stocker, Zinder and Lederberg (1953) the O-variants of the salmonellae were used in the capacity of microorganisms to be altered. The first group of experiments was performed on the O-variants of the salmonellae, which had lost their H-antigens. After the action on them of phage lysates of a motile culture, the H-antigens of which were different from the H-antigens lost by the given O-variant, motile variants were found in the majority of cases which had H-antigens similar to that previously lost and not to that which was contained in the directing culture.

In the second group of experiments, types of salmonellae were used in which the presence of H-variants was unknown.

Here, the majority of the transduced motile strains had H-antigens corresponding to the donor strain, with the exception of some which, in combination with the O-antigens, were characterized as S. heidelberg, S. paratyphi B., and S. dublin.

Acquisition of motility was observed after the treatment of non-motile O-variants with phage lysates of O-cultures which were also non-motile. Flagellar antigens were also acquired in parallel with this.

Kaufman (1953) performed 100 experiments in altering the antigenic structure of salmonellae; in 73 of them a positive result was obtained. Here, as in the work of Stocker, Zinder and Lederberg, the altered microorganisms simultaneously acquired motility and flagellar antigens. In addition, Kaufman described a case of simultaneous alteration of the "H" and the "O" antigens.

Groman and Freeman and others published a series of studies (Freeman, 1952; Freeman and Morse, 1952; Groman, 1953,

1955; Groman and Eaton, 1955) in which a conversion is described of non-toxic diphtheria cultures obtained from a single cell into virulent and toxic cultures under the influence of mild-acting bacteriophage isolated from a lysogenic virulent culture.

The experiments of Parsons and Frobischer give evidence of the possibility of acquisition of virulence by avirulent diphtheria microorganisms under the influence of bacteriophages isolated from avirulent cultures (Parsons and Frobischer, 1951, 1953; Parsons, 1955).

Baron (1953) published data on the alteration of the fermentative activity of salmonellae of various types containing and not containing Vi-antigen under the influence of Vi-bacteriophages. The altered microorganisms did not become lysogenic.

Aside from the work presented above, there are a number of reports in the literature concerning a directed alteration under the influence of phage lysates of somatic antigens, mobility, virulence and a number of other properties (Baron, Formal and Spilman, 1953; Felsenfeld, 1954; Edwards and others, 1955; Narbutovich, 1955; Uetake and others, 1955; Bailey, 1956; Baron, Formal and Washington, 1957).

In the reaction of transformation the phenomenon of transduction primarily approximates the fact that in either case the sterile substrate which has no living bacterial cells possesses transforming activity. In one cell, in the majority of cases, a single feature is altered but in different bacteria in the population different features are changed. New properties acquired as the result of a transduction reaction as well as after the action of DNA are stable and are transmitted hereditarily.

It is well known that DNA of phage is synthesized basically from the elements of the medium assimilated by the cell after infection. Thus, Cohen (1947, 1948) in experiments with bacteriophages T_2 and T_4 showed that after the infection of bacteria tagged with radioactive phosphorus in a medium free of the latter, the phage contains approximately 80 percent of the phosphorus of the medium. When the experiment was performed in a reverse manner, where normal bacteria was infected with the phage in a medium with P^{32}, the radioactivity of the virus phosphorus amounted to 75 percent.

Similar data were obtained by Kozloff and Putnam (1950, 1948) with respect to bacteriophage T_6. They showed that 70 percent of the DNA phosphorus of this phage comes from the medium.

The remaining portion of the phosphorus of the DNA of the virus is obtained from the material present in the cell at the time of the infection. The experiments of Kozloff and Putnam, performed on differentially tagged bacterial cells, attest to the fact that approximately 30 percent of the phosphorus of bacteriohage T_6 comes from the host cell. The results obtained by them give us the basis for believing that DNA of the phage is formed from the DNA of the host.

Kozloff, Nolton, Putnam and Evans (1951) in experiments with E. coli tagged with P^{32} and N^{15} established the fact that 16-43 percent of the DNA of the T_6 phage, which is distributed among all the newly formed phage corpuscles, has a bacterial origin.

Based on the data presented above concerning the participation of the bacterial DNA in the synthesis of the nucleic acid of thephage, it is believed that part of the DNA of the host enters into the composition of the phage without losing specificity, and is injected into the bacterial cell with a subsequent infection. The direct transmission of the DNA of the bacteria to the phage experimentally has not been confirmed. On the other hand, it has been shown that under the influence of nucleic acid of the phage the desoxyribonuclease of the bacterial cell is activated, which produces a degradation of its DNA. The fragments of the latter are broken down to nucleosides, after which they are used for the synthesis of the DNA of the virus. However, taking into consideration the extremely low concentrations of the solutions possessing transforming activity in in vitro experiments (1:600 million), it is apparently impossible to exclude completely the possible significance of the direct transmission of such an insignificant quantity of bacterial nucleic acid, which cannot be determined by the methods of investigation used.

In the process of reproduction of the bacteriophage not only the DNA is used but also the protein components of host cells. Thus, Kozloff and co-authors (Kozloff, Nolton, Putnam, Evans, 1951) showed that five to 25 percent of the protein of the bacterial cell goes into the formation of the progeny of the phage.

Thus, data obtained in experiments on the elucidation of the mechanism of the phenomenon of transduction, like results of investigations on the study of the nature of components transmitted from the original phage particles to the progeny, do not introduce sufficient clarity into the problem of the genetic role of desoxyribonucleic acid.

As is well known, in the experiments on the transformation of microorganisms under the influence of bacterial extracts containing chiefly DNA, even the most purified active substrates contain no less than 0.02 percent protein, the role of which remains unclarified to date. As seen from the data presented above, the significance of the protein component remains unclarified also in the case of reproduction of the transduction phenomenon. In addition, in the very phenomenon of transduction there are many factors which are still unexplained, and the conclusions drawn on the basis of the study of it are often contradictory.

Thus, while in the reproduction of the transformation phenomenon the properties of the donor strain are usually transmitted to the recipient strain, in transduction in certain cases features arise in the modified culture which were absent in the bacteria, the phage lysate of which was used as a directing substrate.

For example, in the investigations of Stocker and coauthors and of Kaufman which have been mentioned on the modification of the antigenic structure of salmonellae, the acquired antigens, in the majority of cases, did not correspond to the antigens of the donor strain. When microorganisms which had previously lost their antigens were used in the capacity of a culture to be modified, the formation specifically of these antigens was observed, antigens which were absent in the directing culture.

While in the experiments of Freeman and Groman and others the avirulent non-toxigenic cultures of diphtheria microorganisms became virulent under the influence of the phage isolated from a virulent culture, the data of Parsons attest to the possibility of obtaining virulent cultures under the influence of phage lysate from avirulent microorganisms.

In almost all cases the phenomenon of transduction is reproduced by means of so-called mid-acting bacteriophages isolated from lysogenic microorganisms. As is known, by the term lysogenicity is understood the carriage of phage by microbial cells in a non-infectious non-pathogenic for

(prophage) a d their capacity to liberate bacteriophage under certain conditions, the lytic and lysogenizing activity of which is expressed only with respect to the other strains of a given species, but which does not lyse the original lysogenic culture. The mild-acting phages transfer the features of bacteria sensitive to their action to the resistant bacteria. The change of the properties of the microorganisms is accompanied by lysogenization (L'vov, 1953; Margarita Lieb, 1955; Boyd, 1956).

On the basis of the genetic experiments on the transfer of prophage-linked features, the conclusion has been drawn that the latter are located on the chromosome, which produces changes in the properties of the microorganisms. However, Baron described experiments on the alteration of the properties of microbial cells by means of ordinary virulent bacteriophages, where the altered microorganisms did not become lysogenic.

In Hershey's opinion (1957), the production of toxin in C. diphtheriae and of antigens in certain salmonellae does not occur as the result of transduction, by which he understands a transfer of genetic material from the bacteria in which the last development cycle occurred, but rather as the result of infection by phage (lysogenization).

It must be taken into consideration that in the reproduction of thephenomenon of transduction, apart from the possible significance of the transfer of the bacterial nucleic acid, processes occurring in the bacterial cells in consequence of the injection of the components of bacteriophage are of definite importance.

Only individual strains of mild-acting phages possess transductive activity. Thus, in the majority of studies made in the institutes of various countries, strains of mild-acting phages $PLT_2 2$ and lambda were used.

Thus, there are more unclarified factors in the mechanism of the transduction phenomenon than in the transformation reaction, produced by means of biologically active bacterial extracts.

After analyzing the data existing in the literature concerning the mechanism of reproduction of bacteriophages and the transformation of microorganisms, Hershey (1956) believes it possible to consider them only as a supplementary

proof of the genetic role of DNA, giving it, however, great significance as the basis of chemistry in genetics.

Up to the present time, there are still no experimental data which can be considered as unconditional proof of the genetic role of desoxyribonucleic acid.

Apart from the fact that the role of the protein component remains unclarified in the transforming activity of bacterial extracts, a number of factors usually regarded as proof of the genetic role of DNA are also far from being indisputable.

Thus, Hotchkiss (1955) points out that the coincidence of the mutagenic spectrum of action of ultra-violet rays with the absorption curve of nucleic acids in the ultra-violet range cannot serve as unconditional proof of the direct participation of DNA in the occurrence of mutations, because the mutagenic effect of the rays is also significantly expressed in the field of the absorption maximum. In addition, in the event of absorption of ultra-violet rays by the nucleic acids or proteins the activated molecules may produce changes of the hereditary properties not directly but rather by acting indirectly on some other components of the microbial cell.

More and more data is being accumulated which attest to the possible genetic significance of ribonucleic acids (RNA). The role of RNA in the synthesis of proteins and particularly proteins of the bacterial cell have been shown by the numerous investigations of the Brashe school (1955, 1957).

It has been established that the proteins may be synthesized by disrupted cells, and in the isolated structural components of cells (Gail, 1956; Spiegelman, 1956, 1957).

It has been shown by Kresin (1957) that the synthesis of a certain number of proteins may be accomplished in systems obtained through the destruction of cell granules consisting of protein and ribonucleic acid, in the presence of amino acids and certain co-factors.

Data on the role of RNA in the synthesis of adaptive enzymes (Gail, 1956) are very interesting. The stoppage of synthesis of RNA markedly inhibits the formation of enzymes. When azaguanine (instead of guanine) is introduced into the RNA molecule the synthesis of adaptive beta-galactosides is stopped. Introduction of azaguanine into the RNA molecule

of the tobacco mosaic virus produces a loss of infectivity, just as the introduction of structural analogues of thymine does when introduced into the RNA molecules of bacteriophages.

It is believed that the RNA molecules fulfill the role of a pattern in the synthesis of enzymes. However, it is assumed to carry out investigations similar to the investigations on the extraction of the transforming factor.

It is not known whether specific RNA corresponds with each enzyme. A number of factors speak for the presence of such a specificity: the acceleration of the synthesis of catalase by the addition of RNA of a homologous species, the existence of enzymes the synthesis of which depends on stable and unstable RNA and others (Gail).

In the investigations of Spirin distinct differences have been shown in the RNA content of gram-positive and gram-negative bacteria with the preservation of the constancy of the nucleotide content of the total RNA during various growth periods. The nucleotide content of the total RNA is altered in the process of mutation, which has been shown by a comparative study of the regenerated filtrable forms and of the original colon group of bacteria.

The most interesting data attesting to the genetic role of RNA were obtained in investigations with plant viruses (Fraenkel-Conrat, 1956; Fraenkel-Conrat and others, 1957; Gierer and Schramm, 1956; Schramm, 1957).

Fraenkel-Conrat isolated crude protein preparations and nucleic acid from the crystalline tobacco mosaic virus. On combining the components obtained, 10-15 percent infectivity is recovered. When utilized in the capacity of the original viruses from which the components mentioned above were obtained, strains which produced various disease symptoms, the newly formedhybrids produced disease with symptoms characteristic of the strain from which the nucleic acid was obtained. The protein was similar to the protein of the donor strain of the protein component. However, the progeny of the hybrid both immunologically and in the amino acid content of the protein was identical with the donor virus of the nucleic acid.

Gierer and Schramm showed that when the RNA of the tobacco mosaic virus was injected into plant cells, virus progeny are formed, and here its protein component proceeds from the plant

cells. The nucleic acid of the virus here, in their opinion, serves as the matrix on which a specific regulation of the amino acids occurs.

In the investigations of Frankel-Conrat, Gierer and Schramm, the significance of the residual infectivity of the RNA remains disputable (0.1 to one percent of the original), which is considered by the authors to be due to the infectivity of the nucleic acid itself. However, the genetic role of some components of the plant viruses deprived of their desoyribonucleic acid is indubitable.

The material presented above permits us to conclude that the genetic function of the DNA does not exhaust the mechanism of control of hereditary properties of microorganisms, which apparently is accomplished by a more complex system of compounds.

The facts obtained in the study of the transformation reaction cannot always be explained from the point of view of genetic laws. The worthlessness of the handling of experiments, well known by the name of the phenomenon of gene-recombination in the light of the data on the transforming activity of DNA, and certain other factors speak for the presence of a conflict between classic genetics and the phenomena of transduction and transformation (Lindergren, 1955).

Investigations on the transformation and transduction of microorganisms undoubtedly attest to the presence of certain biochemical structures--genetic components, under the influence of which the hereditary signs can be modified.

These components, however, do not exhaust the compounds localized in nuclei, and they are not limited to desoxyribonucleic acid.

Control of the hereditary features is accomplished also by cytoplasmic substances, particularly ribonucleic acid.

Furthermore, we believe that the specific action of the transforming extract is only a particular case among the most varied causes producing an alteration of the hereditary properties of microorganisms.

The investigations on the transformation of bacteria carried out by our co-workers and on the study of the processes of formation of filtrable and L-forms of bacteria have quite obviously established the fact that the change in the here-

ditary properties of microorganisms can be obtained through the influence of various factors of the environment: through the action of antibacterial agents and, particularly of antibiotics, under favorable environmental conditions, etc.

It has been shown that under the influence of environmental conditions qualitative changes occur in the components of the cells to which a genetic role has been given.

Specifically, this pertains to the qualitative changes of DNA manifested in a change in the nucleotide content of its molecules.

Thus, in the investigations on the immunochemical study of variability the altered variants were obtained through the influence of non-specific effects (prolonged passage in distilled water, passage through media with low pH, action of antibiotics).

As has been mentioned, marked qualitative changes in the nucleotide content of the DNA have been established in these variants in comparison with the original cultures, which has been correlated with a marked change of their biological properties and of the antigenic structure of the protein fractions.

All these data undoubtedly attest to the fact that certain biochemical structures play an important role in the determination of definite hereditary features and properties of the microbes. Investigations in the direction of the study of these structures have exceptionally great and important significance.

It is quite obvious, however, that the individual components do not determine the entire conglomeration and entire variety of rules and regulations of heredity and its variability. This is why, along with the study of the individual biochemical structures of the microbial cell which have various chemical expressions, investigations are needed for the clarification of their complex significance in interaction and in indisruptible association with the study of the entire variety of biological manifestations of heredity and variability.

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